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RESEARCH PAPER

# Chitosan: Biocontrol agent of *Fusarium oxysporum* in tomato fruit (*Solanum lycopersicum* L.)

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## Abstract

Synthetic fungicides have experienced a significant increase in recent years, necessitating the search for more sustainable and environmentally friendly alternatives. In this regard, chitosan has emerged as an option to reduce reliance on these products. This study evaluated the effect of chitosan as a biocontrol agent against *Fusarium oxysporum* in tomato fruits. A fully randomized experimental design incorporating 6 treatments was employed, consisting of four chitosan treatments (0.5, 1, 2, and 3 g $L^{-1}$), a negative control involving the application of a synthetic fungicide, and a positive control inoculated with *F. oxysporum*. Samples were taken from infected tomato fruits. The F4 isolate of *Fusarium* sp. was identified as *F. oxysporum*, and demonstrated the highest level of virulence. Among the four chitosan treatments, the 3 g $L^{-1}$ treatment showed the highest a percentage of mycelial growth inhibition (PMGI) at 79.92% and the greatest reduction in biomass at 0.65 g, which did not differ significantly from the synthetic fungicide. Regarding disease severity and incidence, there were significant variations among each of the chitosan treatments, with the highest results obtained with the 2 and 3 g $L^{-1}$ treatments. All chitosan treatments reduced disease severity in tomato fruits. Applying chitosan on fruits of the tomato plant presents an alternative for diminishing reliance on synthetic fungicides.

## Keywords

postharvest, antifungal activity, severity, incidence, phytopathogen

## Introduction

The cultivation and consumption of tomatoes (*Solanum lycopersicum* L.) are widespread, making them one of the most important vegetables globally (Safari et al. 2021). Tomato production in Ecuador shows great potential, with an area of 2579 ha in 2020, yielding approximately 14.9 t $ha^{-1}$ (FAOSTAT 2024). Tomatoes are prone to significant losses during the postharvest stage, with reported losses ranging from 17 to 26% (Emana et al. 2017).

Tomatoes are susceptible to fruit rot caused by *Fusarium* spp. in postharvest conditions (Sun et al. 2023). The presence of this disease not only shortens the postharvest life of the crop but also significantly reduces its market value (Safari et al. 2021). This scenario highlights the need to effectively address challenges associated with tomato postharvest to ensure its quality and availability in the market (Godana et al. 2023). The main method of controlling *Fusarium* spp. involves synthetic fungicides, including carbendazim, chlorothalonil, mancozeb, among others (Torres-Rodriguez et al. 2022b; Ismaila et al. 2023). However, the persistent application of such synthetic fungicides has detrimental effects on human health and the environment because of the accumulation of chemical residues and the increase in pathogen resistance (Burandt et al. 2024).

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Chitosan has been identified as an alternative to synthetic fungicides because of their biodegradability and bioactivity properties (Torres-Rodriguez et al. 2021). Chitosan is a natural compound found in shellfish and is commercially produced from their shells (Mohan et al. 2022). Chitosan has been used for phytopathogen control in postharvest (Wang et al. 2020). Regarding its antifungal activity, there are several mechanisms of action of chitosan (Zhang et al. 2024). Chitosan exhibits a polycationic nature, which modifies the permeability of the cytoplasmic membrane by interacting with negatively charged components on the cell surface (Sun et al. 2021). Additionally, chitosan induces defensive responses in both plants and fruits, such as the generation of reactive oxygen species, activation of lytic enzymes, production of pathogenesis-related (PR) proteins, and synthesis of phytoalexins, among other mechanisms (Singh et al. 2022; Suwanchaikasem et al. 2023; Gong et al. 2024).

Previous research supports the efficacy of chitosan in managing decay after postharvest in a range of fruits and vegetables, such as pomegranates (*Punica granatum* L.), strawberries (*Fragaria ananassa* L.), potatoes (*Solanum tuberosum* L.), grapes (*Vitis vinifera* L.), and apples (*Malus domestica* L.) (Munhuweyi et al. 2017; Lateef et al. 2023). These findings underscore the versatility of chitosan as a biocontrol agent postharvest, supporting its potential application in preserving various agricultural products.

## Materials and methods

### Isolation of *Fusarium* spp.

Tomato fruits showing symptoms of fruit rot caused by *Fusarium* spp. were collected in August 2023. The samples were collected from the greenhouse of the Faculty of Agricultural Sciences, Technical State University of Quevedo, at the experimental campus "La María", located at kilometer 7.5 on the Quevedo–El Empalme road. The geographical coordinates are 01°06'24"S, 79°29'70"W, at an altitude of 75 m above sea level.

The samples were placed in sterile plastic bags and transported to the microbiology facility on the "La María" campus for processing. Tomato fruits were disinfected for 30 s with 2% sodium hypochlorite. Subsequently, they were rinsed three times with sterile distilled water and allowed to dry on absorbent paper. The 5-mm fragments of tomato fruits were plated on Petri dishes containing potato dextrose agar (PDA, Difco 39 g $L^{-1}$) supplemented with ampicillin (0.1 g $L^{-1}$) and incubated for 7 days at 28 °C. Colonies were purified by transferring a single spore to Petri dishes with PDA.

### Morphological identification

Morphological identification of *Fusarium* spp. isolates was conducted by evaluating their macroscopic characteristics, such as color, sporulation, mycelium type, and microscopic characteristics, including macroconidia, microconidia, and chlamydospores, following the taxonomic keys of Summerell et al. (2003).

### Pathogenicity of isolates

Fungi were cultured on PDA at 28 °C for 7 days. Spores were collected by scraping with a spatula and sterile distilled water, adjusting the concentration to $1 \times 10^6$ conidia $mL^{-1}$. Tomato fruits were disinfected by immersion in 2% sodium hypochlorite for 3 min, followed by rinsing with sterile distilled water and drying. Then, 2-mm wounds were made on each fruit, and 10 µL of each isolated fungus was inoculated into each wound. A control group was established in which fruits were inoculated with sterile distilled water. The fruits were stored in plastic bags (18 cm × 26 cm × 0.05 mm) placed inside commercial corrugated cardboard boxes (30 cm × 25 cm × 15 cm). These boxes were stored at a temperature of 26 °C and a relative humidity of 60% for 7 days. At the end of this period, the lesion diameter was measured using a digital caliper (Traceable® Digital Calipers), and the fresh and dry weights of the fruits were determined. Disease incidence (ID%) was calculated.

$$ID = (\text{Infected fruits}/\text{Total fruits}) \times 100\%$$

where: Infected fruits are the number of infected fruits, and Total fruits are the total number of fruits.

### Molecular identification and phylogenetic analysis

DNA extraction was performed according to the method of Ochoa et al. (2007). The ITS1-5.8s-ITS2 region of rDNA was amplified using the primers ITS1 (5' TCCGTAGGTGAACCCTGCGG 3') and ITS4 (5" TCCTCCGCTTATTGATATGC 3"). Amplification was performed with a denaturation period of 3 min at 95 °C, followed by 30 cycles (denaturation at 95 °C for 1 minute, annealing for 30 seconds at 50 °C, and extension for 1 min at 72 °C), with a final extension at 72 °C for 10 min. The amplified regions were compared with sequences stored in the NCBI GenBank database using BLAST.

MEGA 7.0.21 software was used for phylogenetic analysis (Kumar et al. 2018). For the construction of the phylogenetic tree, the ITS sequence obtained was aligned with 13 *Fusarium* spp. sequences obtained from NCBI using MUSCLE (Edgar 2004), employing Gblocks (Talavera and Castresana 2007) within the Phylogeny.fr platform (Dereeper et al. 2008) for multiple sequence alignment. Segments with variable positions or gaps were excluded, and conserved blocks were selected. The sequence of *Ectophoma multirostrata* (GenBank Accession: MG238549.1) was selected as the outgroup. The best-fit DNA evolution model was determined using MEGA 7.0.21 software (Kumar et al. 2018). Phylogenetic analysis was performed using the maximum parsimony (MP) method, employing

the Kimura-2 parameter model and Gamma distribution with 10,000 bootstrap replicates. Nearest-Neighbor-Interchange (NNI) was used to obtain the phylogenetic tree.

## Disease severity determination

The severity of tomato fruit disease (DS) was evaluated as described by Mohamed et al. (2017), with modifications. DS of the fruits was assessed on the basis of visible symptoms, spots, rot, and rotten areas on each fruit surface. Four DS scales were used, as shown in Table 1.

**Table 1.** Tomato fruit disease severity scale.

| Severity Scale | Description | Inference |
|---|---|---|
| 0 | No visible symptoms on the fruit | No infection |
| 1 | 1–25% of the area covered by mild necrotic lesions | Mild infection |
| 2 | 26–50% of the inoculated area covered by white and necrotic fungal mycelium | Moderate infection |
| 3 | 51–75% of sample necrotic with the presence of sporulating mass | Severe infection |
| 4 | >76% Necrotic tissue with fungal mass; appears soft and rotten | Very severe/ devastating infection |

## *In vitro* antifungal activity of chitosan against *F. oxysporum*

In the center of Petri dishes (PDA), 5-mm plugs of *F. oxysporum* were placed with different concentrations of low-molecular-weight chitosan (160 kDa, 85% DD) (0.5, 1, 2, and 3 g $L^{-1}$). *F. oxysporum* along with the synthetic fungicide Carbendazim (6 mg $mL^{-1}$) and *F. oxysporum* on PDA were used as controls. All Petri dishes were incubated for 7 days at 28 °C. Mycelial growth was measured when *F. oxysporum* mycelium (control) reached the edges of the Petri dishes using a digital caliper. Percentage inhibition of mycelial growth (PIMG%) of *F. oxysporum* was determined, where PIMG (%) = [(R1-R2)/R1] × 100%, R1 represents the mycelial growth of *F. oxysporum* in the control plate, and R2 is the mycelial growth of *F. oxysporum* in the chitosan treatments.

## Antifungal activity of chitosan on *F. oxysporum* biomass

*F. oxysporum* spore suspension ($1 \times 10^6$ conidia $mL^{-1}$) was introduced into PDB medium with different concentrations of chitosan (0.5, 1, 2, and 3 g $L^{-1}$) and incubated at 28 °C with agitation (170 r/min) for 7 days. Flasks containing *F. oxysporum* in PDB were employed as the control group. Mycelia were filtered through a mesh (pore size, 30 µm), washed with distilled water, and collected by centrifugation at 5,000 rpm (twice). Subsequently, they were dried in an oven and weighed to determine *F. oxysporum* biomass. The experiment consisted of five repetitions per treatment and was repeated twice.

## Antifungal activity of chitosan against *F. oxysporum* in tomato fruit

### *Chitosan treatments as postharvest coating*

Tomato fruit with water prepared with 0.05% sodium hypochlorite was submerged for 3 min before coating treatments. The fruit was rinsed, air-dried for 1 h, and randomly divided into 8 lots. Eight batches of fruit were submerged for 1 min in coating solutions. Six treatments were used, consisting of four chitosan treatments (0.5, 1, 2, and 3 g $L^{-1}$), a negative control consisting of application of a synthetic fungicide, and a positive control inoculated with *F. oxysporum*. All fruits were dried for 2 h at 26 °C and 60% RH. For each coating, six fruits per replicate were used. The fruit was then packed in plastic bags of 18 cm × 26 cm and 0.05 mm. These bags were placed in commercial corrugated cardboard boxes measuring 30 cm × 25 cm × 15 cm. The boxes were then stored at 26 °C and 60% relative humidity for 7 days. The experiment consisted of six repetitions per treatment and was repeated twice with a completely randomized experimental design.

## Evaluation of disease severity in tomato fruits

The severity of tomato fruit disease following chitosan application (DS) was assessed as described by Mohamed et al. (2017), with modifications. The methodology described above was used. Fruits with severity index scores of 2, 3, and 4 were considered to have no commercial or marketing value.

$$DS\ (\%) = [\Sigma^{4}_{i=1}\ ni(sti)/(N \times K)] \times 100$$

where ni = number of fruits in severity stage DS, sti = severity stage value (0–4), N = total number of evaluated fruits, and K = highest scale level (4).

In addition, variables such as damage diameter, fresh weight, and dry weight of tomato fruit were quantified. The experiment consisted of six replicates per treatment and was repeated twice with a completely randomized experimental design.

## Evaluation of disease incidence in tomato fruits

Disease incidence with chitosan application (ID) was measured as the percentage of fruits exhibiting symptoms of rot, according to the method of Khaliq et al. (2015) with modifications. ID was determined as the number of infested fruits showing disease symptoms, such as spots and rot, divided by the total number of fruits. Six tomato fruits were distributed and used for the ID assessment. The percentage of disease incidence was determined using the following formula:

$$\%\ ID = (Fi / TF) \times 100\%$$

where Fi is the number of infected fruits and TF is the total number of fruits. The experiment consisted of six

replicates per treatment and was repeated twice with a completely randomized experimental design.

### Statistical analysis

One-way analysis of variance (ANOVA) was conducted using STATISTICA 10.0 software (StatSoft, Tulsa, OK) to examine the data. The Tukey test ($p \leq 0.05$) was applied to differentiate between means. Before the analysis of variance, data normality was evaluated using the Shapiro–Wilk test, and variance homogeneity was assessed using the Bartlett test. Regarding severity (DS) and disease incidence (ID) in the in vivo experiments, data were analyzed independently using the non-parametric Kruskal–Wallis test because the assumptions of normality and variance homogeneity were not met, even after applying logarithmic, arcsine, or square root transformations. The Dunn comparison test was employed to compare mean values ($p \leq 0.05$).

## Results

### Macroscopic and microscopic characteristics of *Fusarium* sp.

Four isolated strains, designated as F1, F2, F3, and F4, of *Fusarium* spp. were identified and extracted from tomato fruits with rot symptoms. These isolates exhibited variabilities in pigmentation, colony diameter, sporulation, and mycelial type (Table 2).

Distinctive microscopic characteristics of *Fusarium* sp. were identified in the isolates collected from tomato fruits. The isolates exhibited variations in the characteristics of their macroconidia, microconidia, and the presence of chlamydospores. Structures such as macroconidia showed 3, 4, and 5 septa, straight and semicurved shapes, and differences in the apical and basal cells. The microconidia exhibited both reniform and fusiform shapes, with varying numbers of septa ranging from 0 to 1 (Table 3).

### Pathogenicity of *Fusarium* spp.

The four isolates of *Fusarium* spp. (F1, F2, F3, and F4) proved to be pathogens, inducing a 100% disease incidence with varying degrees of severity in the fruit, as detailed in Table 4. Among the isolates, F4 was the most virulent, manifesting a systemic infection within a 7-day period and causing the greatest damage to the evaluated fruits. The decrease in both fresh and dry fruit weight was evident with increasing severity of the disease (Table 4).

Severity: 0 = No visible symptoms on the fruit; 1 = 1–25% of the area covered by mild necrotic inoculations; 2 = 26–50% of the inoculated area covered by white and necrotic fungal mycelia; 3 = 51–75% of the sample is necrotic with presence of spore mass; 4 = >76% Necrotic tissue with fungal mass; appears soft and rotten. Means with equal letters in the columns not significantly differ according to Tukey ($p \leq 0.05$). ± standard deviation.

The PCR amplification of a 550-base pair fragment was observed from the ITS region of the F4 isolate obtained from tomato fruits. Its sequence exhibited 100% identity with *F. oxysporum* sequences in the NCBI database.

**Table 2.** Macroscopic characteristics of *Fusarium* spp. isolated from tomato fruits.

| Isolate | Color of the colony | Colony diameter (mm) | E | Type of mycelium |
|---|---|---|---|---|
| F1 | Gray | 65.42 ± 1.06 c | + | Scanty aerial mycelium, cream-colored whitefom |
| F2 | Light yellow | 71.31 ± 0.78 b | + | Abundant aerial mycelium, white and cottony |
| F3 | White to cream | 75.12 ± 0.97 a | ++ | Scanty aerial mycelium, white, cottony, and hyaline |
| F4 | White to cream | 75.22 ± 1.11 a | +++ | Dense aerial mycelium, white, cottony, and hyaline |

E: Sporulation: +++ abundant ++ good + moderate. Equal letters in the columns do not significantly differ according to Tukey ($p \leq 0.05$). ± standard deviation.

**Table 3.** Microscopic characteristics of *Fusarium* spp. isolated from tomato fruits.

| Isolate | Macroconidium | | | | Microconidium | | Chlamydospore |
|---|---|---|---|---|---|---|---|
| | Shape | Septum | Apical Cell | Basal Cell | Shape | Septum | |
| F1 | Semicurve | 3 | Papillose | Slightly notched | Reniform, fusiform | 0 | Paired, smooth wall |
| F2 | Straight, thin | 3 | Blunt | Foot-shaped | Reniform | 0–1 | – |
| F3 | Semicurve | 4 | Romo | Slightly notched | Reniform, fusiform | 0 | Simple verrucose |
| F4 | Semicurve | 4–5 | Narrowing | Foot-shaped | Reniform | 0–1 | Paired, smooth wall |

**Table 4.** Impact of *Fusarium* spp. severity on tomato fruit quality.

| Isolate | Severity | Damage diameter (mm) | Fresh fruit weight (g) | Dry fruit weight (g) |
|---|---|---|---|---|
| Control | 0 | 0.00 ± 0.00 a | 119.24 ± 4.18 a | 9.75 ± 2.50 a |
| F1 | 1 | 12.01 ± 5.41 b | 94.35 ± 6.31 b | 7.04 ± 3.19 b |
| F2 | 2 | 14.23 ± 8.70 c | 89.56 ± 4.11 c | 6.25 ± 4.11 c |
| F3 | 3 | 16.57 ± 6.44 c | 88.65 ± 5.18 c | 6.03 ± 4.64 c |
| F4 | 4 | 22.08 ± 4.04 d | 81.34 ± 3.05 d | 5.34 ± 4.11 d |

Phylogenetic analysis showed that the ITS sequences of F4 were associated with other *F. oxysporum* isolates, forming a single group (Fig. 1).

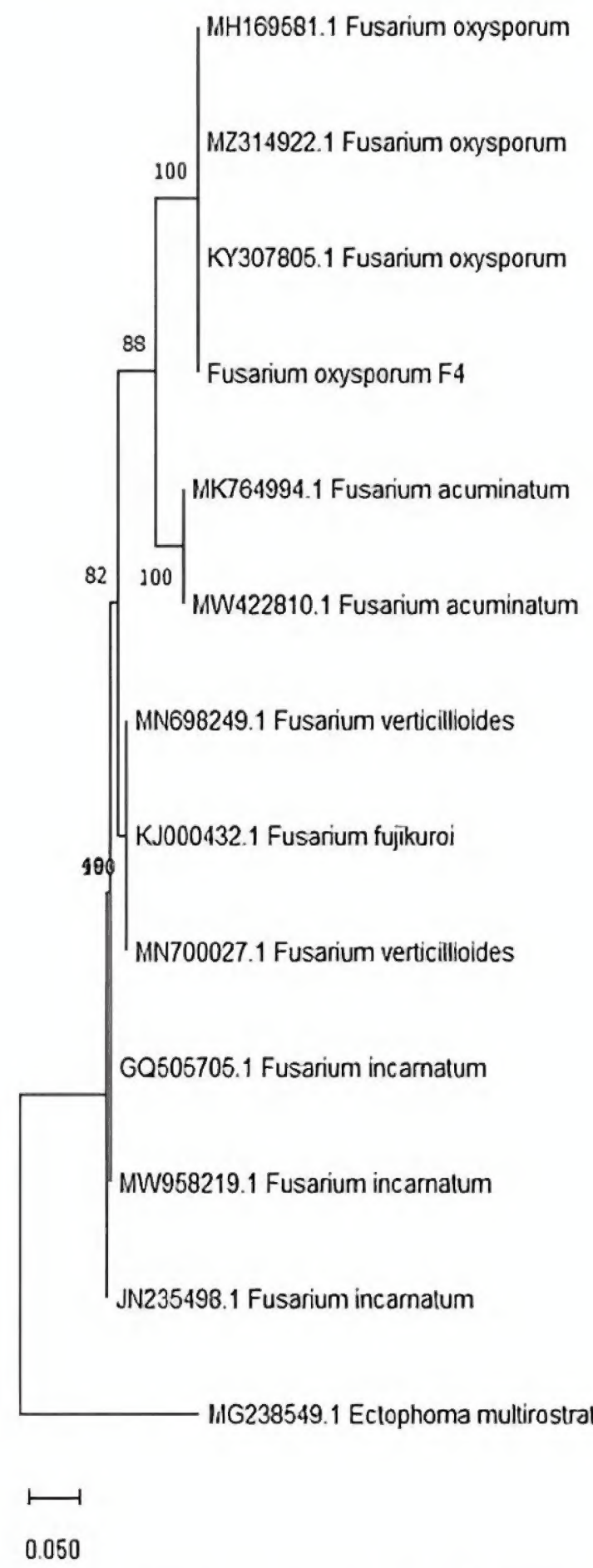


**Figure 1.** Phylogenetic analysis of the F4 fungus isolated from tomato fruit. The GenBank accession number is listed before the names of each species (10,000 bootstrap).

## Antifungal potential of chitosan against *F. oxysporum in vitro*

All four evaluated treatments (0.5, 1, 2, and 3 g $L^{-1}$) exhibited antifungal effects against *F. oxysporum*. Treatment T4 (3 g $L^{-1}$) stood out as the most effective, achieving a mycelial growth inhibition rate of 79.92%, comparable to that of the synthetic fungicide in terms of effectiveness. On the other hand, the 0.5 g $L^{-1}$ treatment recorded the lowest PMGI at 51.51%. The control treatment (*F. oxysporum*) showed no inhibition and was not included in the analysis (Fig. 2).

## Inhibition of *F. oxysporum* biomass by chitosan

Significant variations were observed among the different treatments in the *F. oxysporum* biomass study. In terms of reducing *F. oxysporum* biomass, all four chitosan treatments were effective. Treatment T4 exhibited significant differences compared with T1, T2, and T3 of chitosan, achieving maximum biomass reduction with 0.65 g. No significant differences were observed between treatment T4 (3 g $L^{-1}$) and the synthetic fungicide treatment (Fig. 3).

## Effect of chitosan on tomato fruits inoculated with *F. oxysporum*

*F. oxysporum* treatment exhibited the highest lesion diameter, indicating significant damage compared with chitosan and synthetic fungicide application. Chitosan treatments at 2 and 3 g $L^{-1}$ prevented lesions and rotting in the fruit caused by *F. oxysporum*. The 0.5 g $L^{-1}$ chitosan treatment showed the largest lesion diameter; however, this treatment did not differ significantly from the synthetic fungicide. The application of 3 g $L^{-1}$ resulted in the highest fresh and dry fruit weights, demonstrating significant differences from the other chitosan treatments and Carbendazim (synthetic fungicide). Chitosan treatments were superior to synthetic fungicides (Table 5).

**Table 5.** Effect of chitosan on tomato fruits inoculated with *F. oxysporum*.

| Chitosan treatments | Damage Diameter (mm) | Fresh fruit weight (g) | Dry fruit weight (g) |
|---|---|---|---|
| T1 (0.5 g $L^{-1}$) | 7.33 ± 0.81 b | 116.92 ± 1.36 d | 9.87 ± 0.80 c |
| T2 (1 g $L^{-1}$) | 5.83 ± 0.75 c | 118.65 ± 0.91 c | 9.84 ± 0.79 c |
| T3 (2 g $L^{-1}$) | 0.00 ± 0.00 d | 121.49 ± 1.43 b | 10.23 ± 1.33 b |
| T4 (3 g $L^{-1}$) | 0.00 ± 0.00 d | 124.59 ± 1.55 d | 10.86 ± 1.32 a |
| *Fusarium* sp. | 21.83 ± 1.17 a | 79.28 ± 0.86 a | 6.49 ± 2.03 d |
| Carbendazim | 7.83 ± 0.41 b | 115.88 ± 1.01 d | 9.61 ± 0.51 e |

Means with equal letters in the columns do not significantly differ according to Tukey ($p \leq 0.05$). ± standard deviation.

## Impact of chitosan on *F. oxysporum* incidence and severity in tomato fruits

The application of chitosan on tomato fruits inoculated with *F. oxysporum* resulted in higher efficacy in reducing the disease compared with the use of synthetic fungicide. Effectiveness varied between 0 and 25% for chitosan-treated fruits, showing that each treatment had a significant impact on reducing rot disease caused by *F. oxysporum* compared with the control group (fruits inoculated with *F. oxysporum*). The best treatments in terms of disease reduction were the 2 and 3 g $L^{-1}$ chitosan treatments (Fig. 4).

The incidence of the disease (ID) was lower in tomato fruits treated with chitosan, surpassing the control treatment.

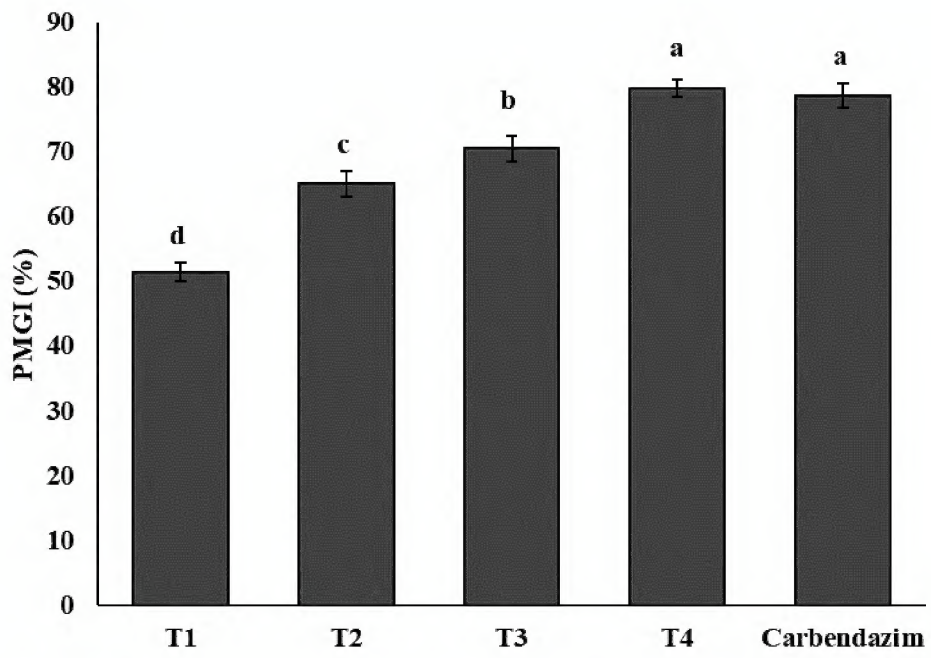


**Figure 2.** Percentage inhibition of mycelial growth (PMGI) of chitosan against *F. oxysporum*. ± standard deviation. Means with equal letters in the columns do not significantly differ according to Tukey ($p \leq 0.05$).

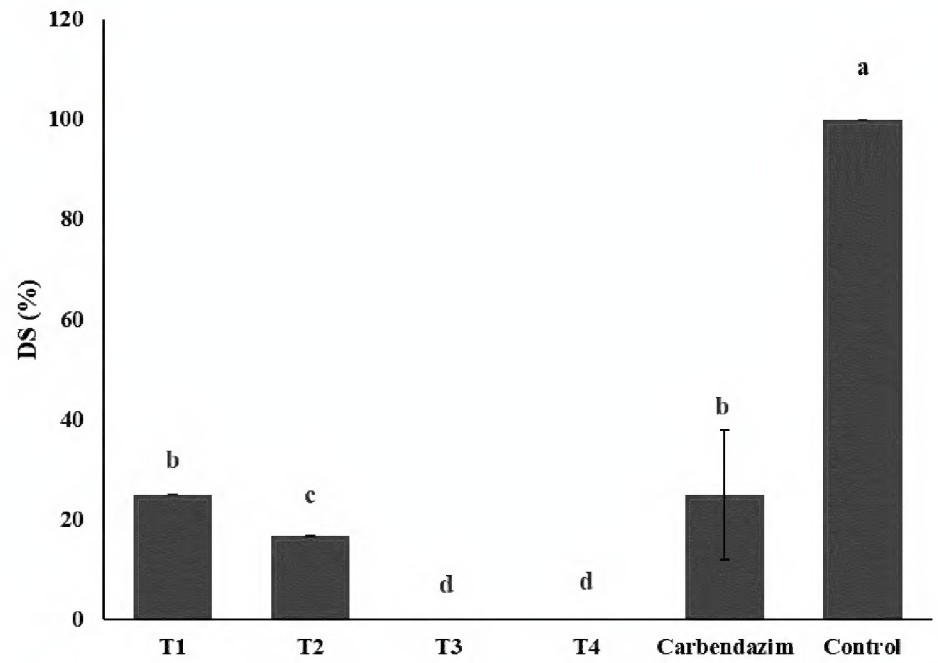


**Figure 4.** Effect of chitosan on disease severity (DS) caused by *F. oxysporum* in tomato fruits. Means with equal letters in the columns do not significantly differ according to Dunn ($p \leq 0.05$). ± standard deviation.

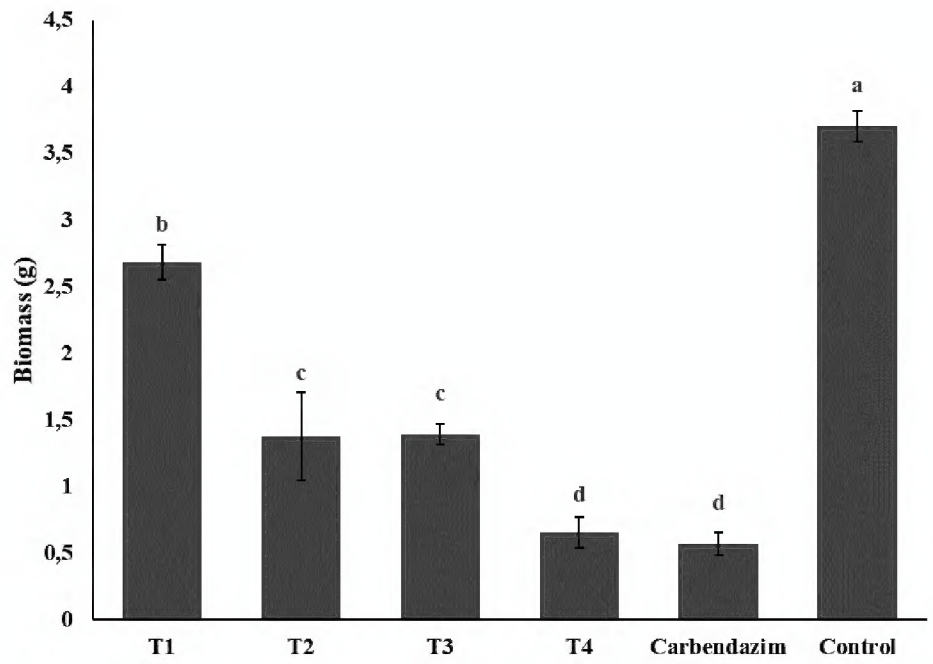


**Figure 3.** Antifungal activity of chitosan against *F. oxysporum* biomass. ± standard deviation. Means with equal letters in the columns do not significantly differ according to Tukey ($p \leq 0.05$).

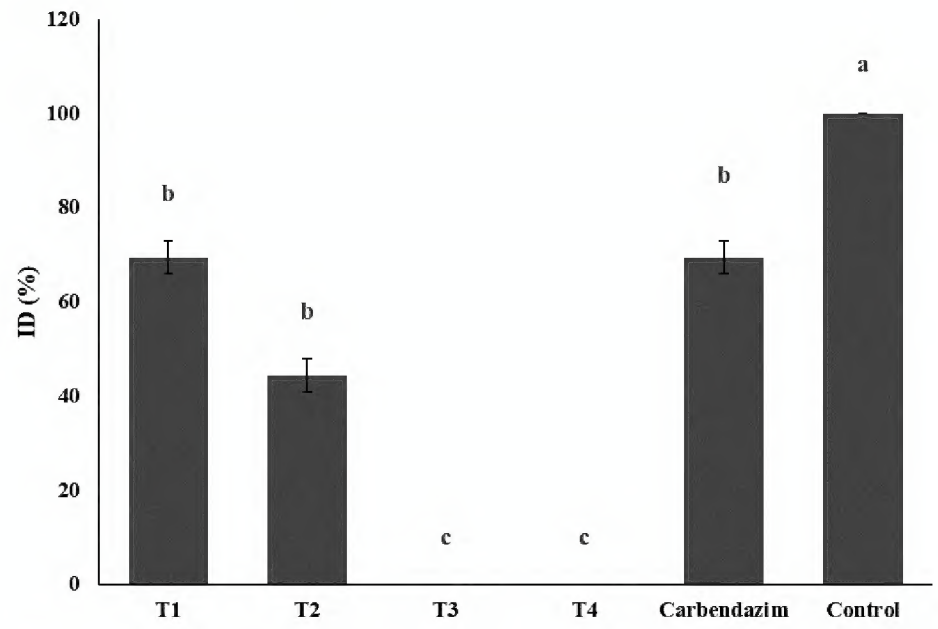


**Figure 5.** Effect of chitosan on the incidence of disease (ID) caused by *F. oxysporum* in tomato fruits. Means with equal letters in the columns do not significantly differ according to Dunn ($p \leq 0.05$). ± standard deviation.

The application of the highest doses of chitosan, 2 and 3 g $L^{-1}$, yielded the best results, showing significant differences compared with the other chitosan treatments and the synthetic fungicide treatment. The treatment without chitosan application (control) resulted in 100% ID in tomato fruits. Chitosan treatments were either superior or equally efficient compared with synthetic fungicide application (Fig. 5).

## Discussion

To achieve effective management of postharvest disease control, accurate isolation and identification of the involved phytopathogenic species are crucial. Four isolates of *Fusarium* spp. were identified from tomato fruits, which exhibited morphological differences. These results confirm the common morphological variability among different *Fusarium* species, as reported by Summerell et al. (2003). Sonkar et al. (2013) observed variations in colony pigmentation among *Fusarium* spp. isolates as well as in colony diameter. Our results mirror the findings reported by Torres-Rodriguez et al. (2022a), who found morphological differences in color, diameter, and type of mycelium among *F. solani* isolates.

Diversity was also reflected in reproduction structures, such as macroconidia, microconidia, and chlamydospores, with differences among the obtained isolates, indicating the possible existence of different species within the *Fusarium* genus. Sonkar et al. (2013) also demonstrated the diversity in macroconidia and microconidia morphology among isolates of *Fusarium* sp. Similarly, Singha et al. (2016) reported variations in the microscopic structures of different *Fusarium* sp. isolates.

Previous research has highlighted variations in virulence among different *Fusarium* spp. isolates, corroborating the complexity of their pathogenicity (Nirmaladevi et al. 2016; Murugan et al. 2020). Van der Does et al. (2019) also found similar findings regarding differences in virulence among different *Fusarium* species in tomato cultivation.

Differences have been demonstrated between the results of morphological identification of *Fusarium* species and molecular identification based on the ITS region. In research by Singha et al. (2016), three isolates were

initially categorized as *F. subglutinans* based on morphological traits but were subsequently classified as *Fusarium* sp. upon analysis of their ITS region. Therefore, molecular biology methods, especially PCR, are important for the correct identification of numerous phytopathogens (Kageyama et al. 2003). In this study, isolate F4 was the most virulent and was identified as *F. oxysporum*.

All chitosan treatments exhibited antifungal activity against *F. oxysporum*. These results support the viability of T4 treatment as an effective option for reducing the need for synthetic fungicides. The antifungal effect of chitosan is based on its ability to interact with components on the cell surface of phytopathogenic fungi, affecting membrane permeability and generating internal osmotic imbalances, resulting in the suppression of mRNA and protein synthesis within fungal cells (Xing et al. 2017; Mauro et al. 2022).

Low molecular weight chitosan reduced mycelial and spore growth of *F. graminearum* compared to the control treatment (Luan et al. 2022). This study agrees with reports by Mejdoub-Trabelsi et al. (2020), who demonstrated that the highest dose (4.0 g $L^{-1}$) of chitosan resulted in an 88.4% reduction of *F. oxysporum*. Results from Zhang et al. (2024) demonstrated chitosan exhibited significant inhibition of *F. solani* mycelial growth.

Chitosan can disrupt fungal metabolic processes, especially those related to energy metabolism, lipids, and sugar, thereby reducing their growth and biomass (Zhang et al. 2024). Chitosan inhibits DNA replication and induces apoptosis in phytopathogen cells (Gong et al. 2024).

Higher doses of chitosan (2 and 3 g $L^{-1}$) inhibited disease progression and the incidence of rot in tomato fruit caused by *F. oxysporum*. Furthermore, treatments 2 and 3 g $L^{-1}$ were superior to synthetic fungicide application. Chitosan creates a semi-permeable coating encasing the fruit, which could impede pathogen proliferation by altering the cell membrane of the phytopathogen, leading to intracellular leakage and eventual cell demise (Khalid et al. 2019).

Moreover, the chitosan layer may improve the fruit's epidermal structure and limit the spread of phytopathogens (Safari et al. 2021). Chitosan coating on strawberries inhibited the proliferation of *Botrytis cinerea*, *Rhizopus stolonifer*, and *Aspergillus niger*, which cause diseases such as gray mold and rot (Melo et al. 2020). Robledo et al. (2018) obtained similar results to this study; the authors demonstrated that chitosan coating on tomato fruits inoculated with *B. cinerea* reduced damage compared with the control treatment.

Chitosan coating may assist in maintaining the integrity of the cell wall against assaults from phytopathogens, potentially delaying infection (Abebe et al. 2017). The results of this study mirrored those documented by Chen et al. (2018), in orange cultivations, which indicated reduced disease incidence and severity in oranges coated with chitosan. Sikder et al. (2019) found that banana fruits treated with chitosan exhibited lower disease incidence and severity, with the highest dose of chitosan yielding the most significant effect.

The application of chitosan to tomato fruit inoculated with *F. oxysporum* controlled the incidence and severity of the disease by more than 70%, results similar to those reported in this study. In addition, the chitosan coating retained the enzymatic activities of phenylalanine ammonia-lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO), while also prolonging the preservation period of tomatoes for 15 days (Safari et al. 2021). The semipermeable layer formed by chitosan on the surface of the fruit can help reduce the respiration rate, minimizing water loss and a decrease in weight, firmness and skin color with delay in the degradation of chlorophyll, prolonging the shelf life of the fruit (Batista Silva et al. 2018).

Studies conducted on tomato fruits inoculated with *B. cinerea*, revealed that the application of chitosan reduced fruit rot by 50% (Karpova et al. 2021). The film created by the increased concentration of chitosan coating might have hindered the proliferation of phytopathogens and delayed the ripening of the tomato fruit, thereby leading to reduced disease incidence occurrence and severity in the fruit.

## Conclusions

Four isolates of *Fusarium* sp. were obtained from tomato fruits, with isolate F4 being the most virulent and causing the greatest damage to the tomato fruits. Isolate F4 was identified as *F. oxysporum*. This phytopathogen caused damage to the tomato fruit compared to the control, highlighting the necessity of exploring alternative methods for its control. All chitosan treatments inhibited *F. oxysporum* under *in vitro* conditions by more than 50%. The 3 g $L^{-1}$ chitosan treatment showed the highest percentage of inhibition of radial growth (PIRG) at 79.92%, which did not differ significantly from the synthetic fungicide. Treatments with concentrations of 2 and 3 g $L^{-1}$ showed the most significant reduction in disease severity and incidence caused by *F. oxysporum*. Chitosan as a biocontrol agent against *F. oxysporum* in tomato fruits is an option for reducing synthetic fungicides.

## Conflicts of interest

The authors declare no conflict of interest.

## Author contributions

Conceptualization, J.A.T.-R.; methodology, J.A.T.-R. and J.J.R.-P.; validation, M.S.C-P.; R.R.G-F. and E.O.R-P.; writing, J.A.T.-R.; review and editing, M.S.C-P.; R.R.G-F.; T.R-G. and J.J.R.-P; visualization M.S.C-P.; R.R.G-F.; T.R-G. and E.O.R-P.; supervision, E.O.R-P. and J.A.T.-R. All authors read and approved the final manuscript.

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# References


Abebe Z, Tola YB, Mohammed A (2017) Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon esculentum* Mill.) fruits. African Journal of Agricultural Research 12(8): 550–565. https://doi.org/10.5897/AJAR2016.11648

Batista Silva W, Cosme Silva GM, Santana DB, Salvador AR, Medeiros DB, Belghith I, da Silva NM, Cordeiro MHM, Misobutsi GP (2018) Chitosan delays ripening and ROS production in guava (*Psidium guajava* L.) fruit. Food Chemistry 242: 232–238. https://doi.org/10.1016/j.foodchem.2017.09.052

Burandt QC, Deising HB, von Tiedemann A (2024) Further limitations of synthetic fungicide use and expansion of organic agriculture in europe will increase the environmental and health risks of chemical crop protection caused by copper-containing fungicides. Environmental Toxicology and Chemistry 43(1): 19–30. https://doi.org/10.1002/etc.5766

Chen C, Cai N, Chen J, Peng X, Wan C (2018) Chitosan-based coating enriched with hairy fig (*Ficus hirta* Vahl.) fruit extract for "Newhall" navel orange preservation. Coatings 8(12): 445. https://doi.org/10.3390/coatings8120445

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard J-F, Guindon S, Lefort V, Lescot M (2008) Phylogeny. fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36: W465–W469. https://doi.org/10.1093/nar/gkn180

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Emana B, Afari-Sefa V, Nenguwo N, Ayana A, Kebede D, Mohammed H (2017) Characterization of pre-and postharvest losses of tomato supply chain in Ethiopia. Agriculture & Food Security 6(3): 1–11. https://doi.org/10.1186/s40066-016-0085-1

FAOSTAT (2024) Datos sobre alimentación y agricultura. http://www.fao.org/faostat/es/ [accessed February 5, 2024]

Godana EA, Yang Q, Zhang X, Zhao L, Wang K, Dhanasekaran S, Mehari TG, Zhang H (2023) Biotechnological and biocontrol approaches for mitigating postharvest diseases caused by fungal pathogens and their mycotoxins in fruits: a review. Journal of Agricultural and Food Chemistry 71(46): 17584–17596. https://doi.org/10.1021/acs.jafc.3c06448

Gong W, Sun Y, Tu T, Huang J, Zhu C, Zhang J, Salah M, Zhao L, Xia X, Wang Y (2024) Chitosan inhibits *Penicillium expansum* possibly by binding to DNA and triggering apoptosis. International Journal of Biological Macromolecules 259(1): 129113. https://doi.org/10.1016/j.ijbiomac.2023.129113

Ismaila AA, Ahmad K, Siddique Y, Wahab MAA, Kutawa AB, Abdullahi A, Zobir SAM, Abdu A, Abdullah SNA (2023) *Fusarium* wilt of banana: Current update and sustainable disease control using classical and essential oils approaches. Horticultural Plant Journal 9(1): 1–28. https://doi.org/10.1016/j.hpj.2022.02.004

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K (2003) Broadly Reactive and Highly Sensitive Assay for Norwalk-Like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR. Journal of Clinical Microbiology 41(4): 1548–1557. https://doi.org/10.1128/JCM.41.4.1548-1557.2003

Karpova N, Shagdarova B, Lunkov A, Il'ina A, Varlamov V (2021) Antifungal action of chitosan in combination with fungicides *in vitro* and chitosan conjugate with gallic acid on tomatoes against *Botrytis cinerea*. Biotechnology Letters 43: 1565–1574. https://doi.org/10.1007/s10529-021-03138-6

Khalid S, Piggot TJ, Samsudin F (2019) Atomistic and coarse grain simulations of the cell envelope of gram-negative bacteria: What have we learned? Accounts of Chemical Research 52(1): 180–188. https://doi.org/10.1021/acs.accounts.8b00377

Khaliq G, Muda Mohamed MT, Ali A, Ding P, Ghazali HM (2015) Effect of gum arabic coating combined with calcium chloride on physico-chemical and qualitative properties of mango (*Mangifera indica* L.) fruit during low temperature storage. Scientia Horticulturae 190: 187–194. https://doi.org/10.1016/j.scienta.2015.04.020

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547. https://doi.org/10.1093/molbev/msy096

Lateef MAA, Hussein HK, Al Qady RAA (2023) The application of chitosan in the preservative for fruits and vegetables and control of post-harvest diseases: a review. European Journal of Agricultural and Rural Education 4(7): 20–26. https://www.scholarzest.com/index.php/ejare/article/view/3736

Luan J, Wei X, Li Z, Tang W, Yang F, Yu Z, Li X (2022) Inhibition of Chitosan with Different Molecular Weights on Barley-Borne *Fusarium graminearum* during Barley Malting Process for Improving Malt Quality. Foods 11(19): 3058. https://doi.org/10.3390/foods11193058

Mauro M, Pinto P, Settanni L, Puccio V, Vazzana M, Hornsby BL, Fabbrizio A, Di Stefano V, Barone G, Arizza V (2022) Chitosan film functionalized with grape seed oil—Preliminary evaluation of antimicrobial activity. Sustainability 14(9): 5410. https://doi.org/10.3390/su14095410

Mejdoub-Trabelsi B, Touihri S, Ammar N, Riahi A, Daami-Remadi M (2020) Effect of chitosan for the control of potato diseases caused by *Fusarium* species. Journal of Phytopathology 168(1): 18–27. https://doi.org/10.1111/jph.12847

Melo NFCB, de Lima MAB, Stamford TLM, Galembeck A, Flores MAP, de Campos Takaki GM, da Costa Medeiros JA, Stamford-Arnaud TM, Montenegro Stamford TC (2020) *In vivo* and *in vitro* antifungal effect of fungal chitosan nanocomposite edible coating against strawberry phytopathogenic fungi. International Journal of Food Science & Technology 55(11): 3381–3391. https://doi.org/10.1111/ijfs.14669

Mohamed NTS, Ding P, Ghazali HM, Kadir J (2017) Biochemical and cell wall ultrastructural changes in crown tissue of banana (*Musa* AAA 'Berangan') fruit as mediated by UVC irradiation against crown rot fungal infection. Postharvest Biology and Technology 128: 144–152. https://doi.org/10.1016/j.postharvbio.2017.02.004

Mohan K, Ganesan AR, Ezhilarasi PN, Kondamareddy KK, Rajan DK, Sathishkumar P, Rajarajeswaran J, Conterno L (2022) Green and eco-friendly approaches for the extraction of chitin and chitosan: A review. Carbohydrate Polymers 287: 119349. https://doi.org/10.1016/j.carbpol.2022.119349

Munhuweyi K, Lennox CL, Meitz-Hopkins JC, Caleb OJ, Sigge GO, Opara UL (2017) Investigating the effects of crab shell chitosan on fungal mycelial growth and postharvest quality attributes of pomegranate whole fruit and arils. Scientia Horticulturae 220: 78–89. https://doi.org/10.1016/j.scienta.2017.03.038

Murugan L, Krishnan N, Venkataravanappa V, Saha S, Mishra AK, Sharma BK, Rai AB (2020) Molecular characterization and race identification of *Fusarium oxysporum* f. sp. *lycopersici* infecting tomato in India. 3 Biotech 10(486): 1–12. https://doi.org/10.1007/s13205-020-02475-z

Nirmaladevi D, Venkataramana M, Srivastava RK, Uppalapati SR, Gupta VK, Yli-Mattila T, Clement Tsui KM, Srinivas C, Niranjana SR, Chandra NS (2016) Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. Scientific Reports 6(21367): 1–14. https://doi.org/10.1038/srep21367

Ochoa JL, Herná-Montiel LG, Latisnere-Barragán H, León de La Luz JL, Larralde-Corona CP (2007) Isolation and identification of pathogenic fungi from orange *Citrus sinensis* L. Osbeck cultured in Baja California Sur, Mexico. Ciencia y Tecnología Alimentaria 5(5): 352–359. https://doi.org/10.1080/11358120709487712

Robledo N, Vera P, López L, Yazdani-Pedram M, Tapia C, Abugoch L (2018) Thymol nanoemulsions incorporated in quinoa protein/chitosan edible films; antifungal effect in cherry tomatoes. Food Chemistry 246: 211–219. https://doi.org/10.1016/j.foodchem.2017.11.032

Safari ZS, Ding P, Nakasha JJ, Yusoff SF (2021) Controlling *Fusarium oxysporum* Tomato Fruit Rot under Tropical Condition Using Both Chitosan and Vanillin. Coatings 11(3): 367. https://doi.org/10.3390/coatings11030367

Sikder MBH, Islam MM (2019) Effect of shrimp chitosan coating on physico-chemical properties and shelf life extension of banana. International Journal of Engineering Technology and Sciences 6(1): 41–54. https://doi.org/10.15282/ijets.v6i1.1390

Singh RK, Ruiz-May E, Rajput VD, Minkina T, Gómez-Peraza RL, Verma KK, Shekhawat MS, Pinto C, Falco V, Quiroz-Figueroa FR (2022) Viewpoint of chitosan application in grapevine for abiotic stress/disease management towards more resilient viticulture practices. Agriculture 12(9): 1369. https://doi.org/10.3390/agriculture12091369

Singha IM, Kakoty Y, Unni BG, Das J, Kalita MC (2016) Identification and characterization of *Fusarium* sp. using ITS and RAPD causing *Fusarium* wilt of tomato isolated from Assam, North East India. Journal of Genetic Engineering and Biotechnology 14(1): 99–105. https://doi.org/10.1016/j.jgeb.2016.07.001

Sonkar P, Kumar V, Sonkar A (2013) Studies on cultural and morphological characters of tomato wilt (*Fusarium oxysporum* f. sp. *lycospersici*). International Journal of Biological Sciences 10: 1637–1640.

Summerell BA, Salleh B, Leslie JF (2003) A utilitarian approach to *Fusarium* identification. Plant Disease 87(2): 117–128. https://doi.org/10.1094/PDIS.2003.87.2.117

Sun X, Jiang S, Hong H, Zhang M, Xin Z, Wu B, Xin X (2023) First report of fruit rot caused by *Fusarium luffae* in cherry tomato in China. Plant Disease 106(6): 1763. https://doi.org/10.1094/PDIS-12-21-2666-PDN

Sun Y, Shang L, Xia X, Meng D, Ren Y, Zhang J, Yao M, Zhou X, Wang Y (2021) Cellular uptake of chitosan and its role in antifungal action against *Penicillium expansum*. Carbohydrate Polymers 269: 118349. https://doi.org/10.1016/j.carbpol.2021.118349

Suwanchaikasem P, Nie S, Idnurm A, Selby-Pham J, Walker R, Boughton BA (2023) Effects of chitin and chitosan on root growth, biochemical defense response and exudate proteome of *Cannabis sativa*. Plant-Environment Interactions 4(3): 115–133. https://doi.org/10.1002/pei3.10106

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164

Torres-Rodriguez JA, Reyes-Pérez JJ, Castellanos T, Angulo C, Quiñones-Aguilar EE, Hernandez-Montiel LG (2021) A biopolymer with antimicrobial properties and plant resistance inducer against phytopathogens: Chitosan. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49(1): 12231–12231. https://doi.org/10.15835/nbha49112231

Torres-Rodriguez J, Reyes-Pérez J, Castellanos T, Angulo C, Quiñones-Aguilar E, Hernandez-Montiel L (2022a) Identification and morphological characterization of marine actinomycetes as biocontrol agents of *Fusarium solani* in tomato. Revista de la Facultad de Agronomía de la Universidad del Zulia 39(1): e223915–e223915. https://produccioncientificaluz.org/index.php/agronomia/article/view/37706

Torres-Rodriguez JA, Reyes-Pérez JJ, Quiñones-Aguilar EE, Hernandez-Montiel LG (2022b) Actinomycete potential as biocontrol agent of phytopathogenic fungi: mechanisms, source, and applications. Plants 11(23): 3201. https://doi.org/10.3390/plants11233201

van der Does HC, Constantin ME, Houterman PM, Takken FLW, Cornelissen BJC, Haring MA, van den Burg HA, Rep M (2019) *Fusarium oxysporum* colonizes the stem of resistant tomato plants, the extent varying with the R-gene present. European Journal of Plant Pathology 154: 55–65. https://doi.org/10.1007/s10658-018-1596-3

Wang W, Xue C, Mao X (2020) Chitosan: Structural modification, biological activity and application. International Journal of Biological Macromolecules 164: 4532–4546. https://doi.org/10.1016/j.ijbiomac.2020.09.042

Xing K, Liu Y, Shen X, Zhu X, Li X, Miao X, Feng Z, Peng X, Qin S (2017) Effect of O-chitosan nanoparticles on the development and membrane permeability of *Verticillium dahliae*. Carbohydrate Polymers 165: 334–343. https://doi.org/10.1016/j.carbpol.2017.02.063

Zhang L, Yang Y, Zhu Y, Hu H, Jia Q, Sun C, Zhu X, Liu Y (2024) Antifungal activity and mechanism of chitosan against *Fusarium solani* caused ginger soft rot during postharvest storage. Postharvest Biology and Technology 208: 112680. https://doi.org/10.1016/j.postharvbio.2023.112680